Review
Future Cardiology

Oxysterols in heart failure

Valeriy Lukyanenko† & Yevgeniya Lukyanenko
†Author for correspondence: Medical Biotechnology Center, University of Maryland Biotechnology Institute, 725 W. Lombard St, Rm S216, Baltimore, MD 21201, USA ■ Tel.: +1 410 706 8559 ■ Fax: +1 410 706 8184 ■ lukyanen@umbi.umd.edu

Oxysterols are biologically active molecules that result from the oxidation of cholesterol. Several oxysterols are found in macrophages and macrophage-derived 'foam cells' in atherosclerotic tissue. Lipophilic oxysterols penetrate cell membranes and, therefore, their concentrations can reach harmful levels in endothelial and smooth muscle cells located in close proximity to the atherosclerotic plaques or inflammatory zones. New findings suggest that the effects of oxysterols on cardiomyocytes can lead to cell hypertrophy and death. This may make oxysterols one of the major factors precipitating morbidity in atherosclerosis-induced cardiac diseases and inflammation-induced heart complications. The pathological actions of oxysterols on muscle cells were shown to depend on dysfunctional Ca^{2+} signaling; however, the mechanisms of the effects remain to be elucidated. Understanding the effects of oxysterols could lead to therapies that modulate malfunction of cardiomyocytes. This review discusses the experimental findings and the relevance of oxysterols to heart failure, and suggests strategies for important future investigations.

Tobacco exposure, a diet high in cholesterol and genetic predisposition to elevated lipids has been linked to premature atherosclerosis, cardiovascular diseases, cardiovascular disability, and death [1,2]. Until now, the primary hypothesis that linked elevated cholesterol to such dysfunction has been vascular, mainly focusing on the development of atherosclerotic changes in the blood vessels. However, new findings suggest that heart muscle can develop dysfunctional Ca^{2+} cycling leading to cell death from elevated levels of serum cholesterol derivatives, the oxysterols, which already were demonstrated to contribute to the development of such diseases as age-related macular degeneration, Alzheimer's disease, and osteoporosis [3].

Macrophages play a central role in atherogenesis due to the accumulation of cholesterol, and the production of inflammatory mediators and cytokines. Recently, macrophages and macrophage-derived 'foam cells' were also demonstrated to produce oxysterols, which can induce apoptosis of macrophages themselves, as well as in neighboring endothelial and smooth muscle cells [4,5]. In advanced lesions, this promotes the development of the necrotic core, a key factor leading to acute luminal thrombosis [6].

When macrophage oxysterols are released directly to coronary arteries, they could also reach harmful levels in cardiac cells. However, the effects of oxysterols on cardiac myocytes remain largely unknown. The purpose of this article is to discuss the possible mechanisms underlying effects of oxysterols in cardiac myocytes. Based on the analysis of effects of oxysterols on smooth muscle cells and our preliminary data concerning effects of oxysterols on Ca^{2+} signaling in ventricular myocytes [7], the article discusses some possible mechanisms of such effects. For detailed reviews of oxysterols, their nomenclature, generation, and biological effects see Brown and Jessup [8], Schroepfer [4], and Vejux *et al.* [3].

Oxysterols

Oxysterols are biologically active molecules generated during the oxidation of cholesterol. There are two mechanisms for oxysterol production: autoxidation and enzymatic oxidation. In cholesterol, the most sensitive positions for autoxidation are 4, 5, 6 and 7 (Figure 1) [3]. It is now widely accepted that oxysterols resulting from enzymatic synthesis are 7α-, 24S-, 25- and 27-hydroxycholesterols [3,4,9].

In the plasma lipophilic oxysterols are transported mainly by low-density lipoproteins (LDLs) and, probably, by albumin [3]. Although cholesterol itself is not toxic [2,10,11], some cholesterol oxides, 7-ketocholesterol, α-TRIOL (cholestan-3β,5α,6β-triol), 7β-hydroxycholesterol, and 25-hydroxycholesterol, have been demonstrated to injure vascular endothelial and smooth muscle cells [12–16]. Notably, 25-hydroxycholesterol is one of the most toxic agent in the group [12,13,17].

25-hydroxycholesterol and 27-hydroxycholesterol were found in alveolar, peritoneal and testicular macrophages and in macrophage-derived 'foam cells' from atherosclerotic tissue [9,11,13,18–20]. Although the mechanisms of

Keywords
atherosclerosis ■ Ca^{2+} signaling ■ cardiac myocyte ■ cholesterol ■ foam cells ■ MAPK ■ mitogen-activated protein kinase ■ ryanodine receptors ■ sarcoplasmic reticulum ■ SERCA

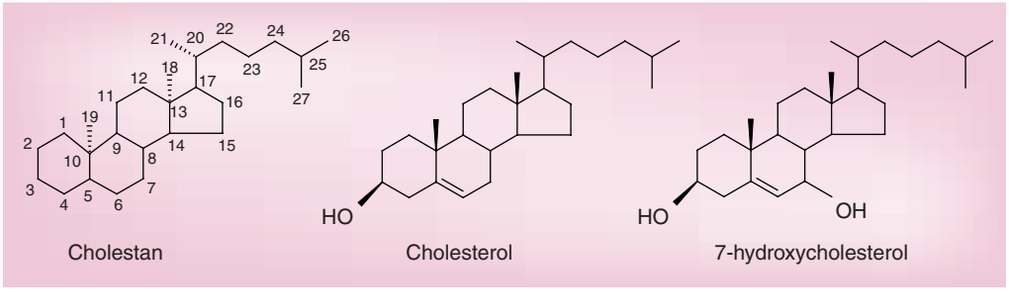

Figure 1. Chemical structures of cholestan (with numbering system) and its derivatives, cholesterol and one of the most toxic oxysterols, 7-hydroxycholesterol. For more structures of oxysterols see [3,8].

accumulation of other oxysterols in 'foam cells' remains mostly unknown, 25-hydroxycholesterol was demonstrated to be produced in macrophages through 25-hydroxylation of cholesterol by 25-hydroxylase [20,21] (for oxysterols in other tissues see [3,4,9,22]). In regard to the production mechanisms of other oxysterols found in 'foam cells', it is important to consider the paper by Zarev *et al.* [23]. The authors demonstrated the weak ability of $\cdot OH/O_2\cdot^-$ free radicals to initiate oxidation of cholesterol in LDLs. This suggests that they could be produced primarily in the same (enzymatic) way as 25-hydroxycholesterol in macrophages.

The reason why macrophages produce oxysterols remains unknown. It has been suggested that such hydroxylation of cholesterol (Figure 1) to oxygenated sterols could serve to remove cholesterol from the cell and transport it to the liver for excretion [4,9,24,25]. However, oxysterols were also shown to regulate multiple physiological functions. For instance, testicular macrophages produce 25-hydroxycholesterol to stimulate steroidogenesis in Leydig cells through a steroidogenic acute regulatory protein-independent pathway [20,26]. Lipophilic oxysterols easily penetrate cell membranes [3,8,9,20,26,27] and, therefore, can diffuse from macrophages (or 'foam cells') into the surrounding endothelial, smooth muscle and cardiac cells.

Reported plasma oxysterol levels in apparently healthy humans depend on sample freshness, assay method and cholesterol levels [4,8,28,29]. In fresh samples levels can vary between tens and hundreds of nanomoles [4]. Increased plasma concentrations of oxysterols were demonstrated to be associated with an increased risk of atherosclerosis, cardiovascular disease, and diabetes [3,4,8,29–31]. Although the major effect of oxysterols is an increase of cytosolic Ca^{2+} concentrations ($[Ca^{2+}]_{cyt}$) [3,14,15,29,32–35], certain underlying mechanisms in cardiac myocytes remain to be elucidated.

Cardiac Ca^{2+} handling

The release of Ca^{2+} from the sarcoplasmic reticulum (SR) is a critical process in the normal physiology of cardiac myocytes. SR Ca^{2+} cycling is a balance between Ca^{2+} release and uptake [36–38]. In adult mammalian cardiac myocytes, the SR serves as the intracellular Ca^{2+} pool for the regulation of cardiac contraction [39,40]. Figure 2 shows the ultrastructure of cardiac myocytes. Ca^{2+} release occurs in the cleft between the SR and sarcolemma of a T-tubule, which is enriched SR Ca^{2+} release channels termed, ryanodine receptors (RyRs). The close proximity of the SR to the sarcolemma suggests that oxysterols could also be involved in the regulation of SR Ca^{2+} cycling.

The initiation of contraction of the cardiac cell depends on Ca^{2+} released from the SR, and the SR Ca^{2+} load depends on the functional state of the major SR Ca^{2+} transporting mechanisms, that is to say of the RyR and SR Ca^{2+}-ATPase (SERCA). Although SR Ca^{2+} cycling is regulated by multiple mechanisms (including magnesium, FKBP12, sorcin, calmodulin, phospholamban, protein kinases, etc.), the major mechanism for Ca^{2+} release from the SR is the so-called Ca^{2+}-induced Ca^{2+} release [37,41–44]: Ca^{2+} entering the cytosol through sarcolemmal Ca^{2+} channels activates the RyRs. Modulation of Ca^{2+} uptake by SERCA or Ca^{2+} leak/release through the RyRs results in corresponding changes in the SR Ca^{2+} load, $[Ca^{2+}]_{cyt}$ and characteristics of spontaneous SR Ca^{2+} release (Figure 3) [38]. Our study on spatio-temporal characteristics of elementary Ca^{2+} release events and Ca^{2+} sparks showed that an increase in $[Ca^{2+}]_{cyt}$ can result from: first, inhibition of SR Ca^{2+} uptake; second, an increase in Ca^{2+} leak from the SR through RyRs; and third, SR Ca^{2+} overload induced by input of extracellular Ca^{2+} [38,45,46].

A Ca^{2+} spark is a bright and short fluorescent signal corresponding to a transient (~10 ms) and local (~1 μm^3) elevation of $[Ca^{2+}]$ that reflects the

activation of greater than 10 RyRs [46–49]. Ca^{2+} sparks can occur spontaneously or be evoked by the activation of Ca^{2+} channels [48,50,51]. Under normal conditions, almost all sparks remain localized and die out without inducing global Ca^{2+} release. Under conditions of increased cellular Ca^{2+} load, sparks increase in amplitude and frequency and become initiation sites of propagating Ca^{2+} waves [45–47,52]. Our data [38,45] demonstrated that changes in the frequency and spatio–temporal characteristics of Ca^{2+} sparks directly reflect changes in SR Ca^{2+} cycling. For example, the activation of RyRs reduces spark frequency and amplitude, and the activation of Ca^{2+} uptake constantly increases the frequency and amplitude. Recording of Ca^{2+} sparks was demonstrated to be a valuable approach for revealing mechanisms underlying Ca^{2+} release effects [53].

Defective SR Ca^{2+} cycling was found to be responsible for defective excitation–contraction coupling in heart failure [54–62] and was demonstrated to lead to a variety of cardiac dysfunctions. For example, spontaneous Ca^{2+} release and increased SR Ca^{2+} leak has been implicated in genetic and acquired triggered arrhythmias and the initiation of ventricular fibrillation during postischemic reflow [60,61,63–71].

We conclude that the data from the literature on arterial smooth muscle cells and our recent experiments in cardiac myocytes suggest a direct inhibitory effect of oxysterols on the SR Ca^{2+} uptake.

Atherosclerosis

Atherosclerosis is a life-threatening disease that affects critical organs including the heart and brain [72]. Atherosclerotic plaques (deposits of fatty substances, cholesterol, cellular waste products, calcium and other substances in the inner lining of an artery) usually affect large and medium-sized arteries, including coronary arteries (coronary atherosclerosis; [73]). Atherosclerosis and its complications, such as coronary heart disease, ischemic heart disease, heart failure, heart infarction and stroke, are the primary causes of death in Western countries [1,74,75].

While the precise steps leading to atherosclerosis remain elusive [2], postulated steps in atherogenesis show a critical role for inflammation in the development of the 'fatty streak', progressive atherosclerotic lesions, and the development of an occlusive thrombus [75–78]. In short, blood monocytes traverse the endothelial intercellular space and are deposited in the subendothelial layers where they are transformed into macrophages. During the various stages of atherogenesis the number of macrophages in the 'fatty streak' could increase due to the expression of various cytokines, which are potent chemoattractants for inflammatory cells including macrophages [76]. The formation of oxysterols owing to the oxidation of the cholesterol-rich LDL accelerates the uptake of cholesterol into macrophages, thereby leading to the next step – the formation of the cholesterol-laden 'foam cell' [12,76,79].

It is notable that oxysterols induce apoptosis of macrophages themselves and in neighboring endothelial and smooth muscle cells [4,5] promoting the development of the necrotic core, a key factor leading to acute luminal thrombosis [6]. Although functional effects of oxysterols on noncardiac muscle cells have been described very well, the underlying mechanisms of the effects and the role of oxysterols in the development of atherosclerosis-related heart failure remain largely unknown.

Heart failure

Heart failure is a syndrome with many different well-described causes, including myocardial infarction, pressure overload, volume overload, viral myocarditis, toxic cardiomyopathy, mutations in genes encoding for sarcomeric or cytoskeletal proteins, and valve diseases [59].

Heart failure develops when the amount of blood pumped from the heart is inadequate to meet the metabolic demands of the body [59,80]. Although the later stages of the disease are well

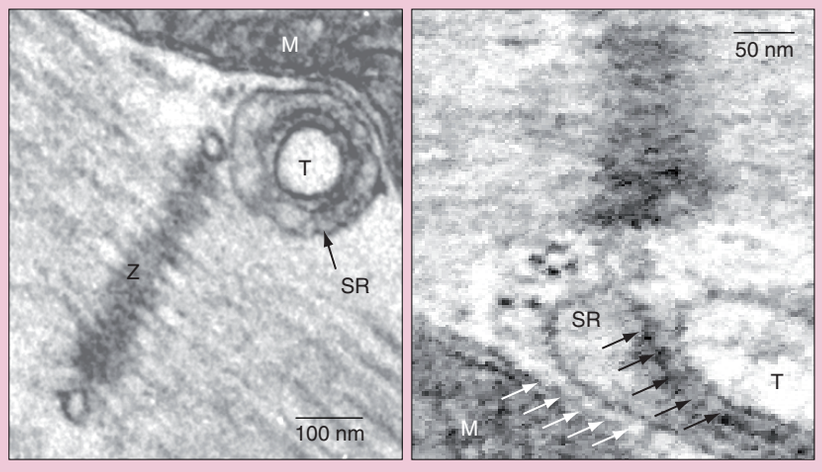

Figure 2. Ultrastructure of ventricular myocyte. The electron micrographs demonstrate peri-T-tubule region in ventricular myocyte. Longitudinal ultrathin section, Epon embedding. Solid arrows show ryanodine receptors (RyRs). White arrows show structures connecting the mitochondrion and the SR.
M: Mitochondrion; SR: Sarcoplasmic reticulum; T: T-tubule; Z: Z-line.
Reproduced with permission from [120].

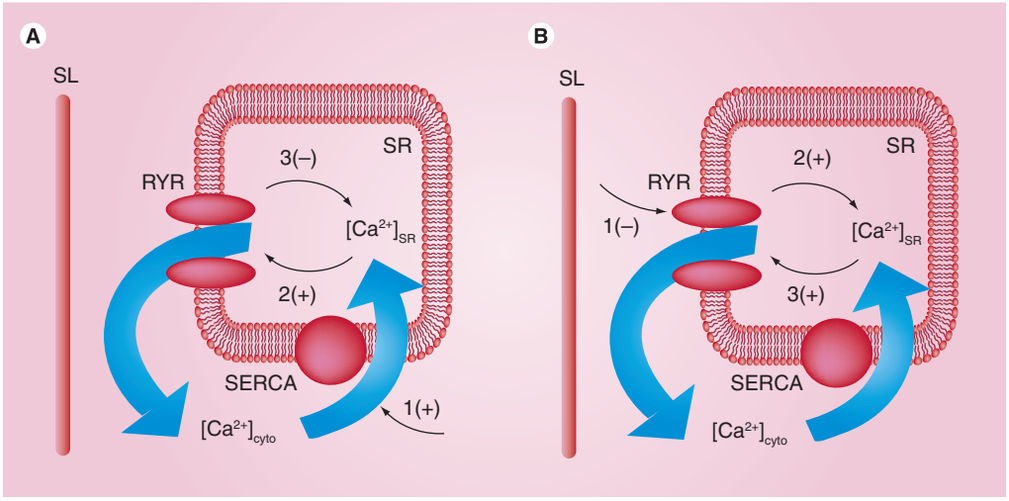

Figure 3. Simplified schematic of dynamic control of SR Ca^{2+}. Ca^{2+} in the SR luminal and cytosolic compartments of the cell during pharmacological interventions either enhancing the activity of the SERCA pump **(A)** or reducing the activity of the RyR **(B)**. **(A)** Stimulation of the SERCA pump increases SR Ca^{2+} ($[Ca^{2+}]_{SR}$) (1) which, in turn, stimulates more Ca^{2+} efflux through RyR channels (2). This enhanced Ca^{2+} efflux reduces $[Ca^{2+}]_{SR}$ (3). **(B)** Inhibition of RyRs (1) decreases Ca^{2+} efflux through RyR channels. This reduced Ca^{2+} efflux increases $[Ca^{2+}]_{SR}$ (2) which, in turn, stimulates the opening of more RyRs (3).
SERCA: Sarco/endoplasmic reticulum Ca^{2+}-ATPase; SL: Sarcolemma; SR: Sarcoplasmic reticulum.
Adapted with permission from [38].

characterized (hypertrophy, and abnormal excitation-contraction coupling and Ca^{2+} handling; for reviews see refs. [59,74]), the starting stages of heart failure are not specifically related to specific pathological processes and are usually characterized just as depressed contractility [80]. In the case of atherosclerosis, the primary mechanism inducing the cascade of events leading to heart failure has not been identified.

Some studies suggest that the primary mechanism could be related to macrophage oxysterols. Blum and Miller [81] and later Tomaselli and Zipes[74] demonstrated that inflammatory processes are directly involved in cardiac depression and in the complex syndrome of heart failure. Also, oxysterols have been demonstrated to be involved in the pathogenesis of the early stages of atherosclerosis [2]. Similar to atherosclerotic complications described for smooth muscle cells, the effect of oxysterols on cardiomyocytes could be mediated by mitogen-activated protein kinases (MAPKs). Indeed, activation of the p38 MAPK was demonstrated to cause deleterious cardiac contractile dysfunction by decreasing myofilament response to Ca^{2+} [82] through downregulation of SERCA expression [83] and/or via p38-dependent production of reactive oxygen species [84]. However, the last statement is contrary to the widely accepted hypothesis that p38 MAPK activation enhances cardiac myocyte survival in response to apoptotic stimuli [85]. This paper suggests that, at least in the case of atherosclerosis-induced heart failure, the onset of dysfunction could be initiated by the direct effects of oxysterols on the mechanisms underlying SR Ca^{2+} cycling and/or by regulating the activity of MAPKs. Indeed, the abnormalities in Ca^{2+} handling during heart failure (such as an increase in resting $[Ca^{2+}]_{cyt}$ [86]) are similar to those caused by oxysterols. Thus, the increasing effect of the oxysterols on cytosolic $[Ca^{2+}]$ could eventually result in MAPK-induced cell death [87].

Oxysterol-induced changes in cell ultrastructure & function

It is now widely accepted that oxysterols have cytotoxic and proinflammatory effects [3,4,34,88]. The quantification of oxysterols in LDL extracts and the comparison with the toxicity of authentic standards indicate that some of them are also present at levels harmful to muscle cells [9,89]. Indeed, oxysterols were demonstrated to have strong effects on the surrounding cells. These effects include: reduction in lipoprotein lipase mRNA and decrease in lipoprotein lipase activity; downregulation of Bcl-2 expression; activation of caspases; degradation of poly(ADP-ribose) polymerase - a substrate for caspase 3 and a key enzyme involved in genome surveillance and DNA repair; production of reactive oxygen species; opening of mitochondrial permeability transition pore; activation of glycogen synthase

kinase-3β; increased synthesis of interleukin-8, which is an attractant for T-lymphocytes; inhibition of proliferation of resting T cells; depletion of thapsigargin-releasable Ca^{2+} stores; activation of MAPKs; fragmentation of DNA; alterations in cell morphology; and cell death [2,3,11,13–16,32,34,77,89–95]. The effects could be divided into two groups: short-term effects on cell physiology and long-term effects that involve changes in cell morphology and lead to cell death.

Effects of oxysterols on cell physiology

Lipophilic oxysterols are able to enter intracellular membranes and affect the structure and function of membrane-associated proteins [8]. In aortic smooth muscle cells 10 μM 7β-hydroxycholesterol and 25-hydroxycholesterol were shown to inhibit activities of Na^+-K^+-ATPase, 5′-nucleotidase, and SERCA [32,96]. Long-term effects of the oxysterols are probably mediated by their influence on Ca^{2+} homeostasis in target cells. Recently Rusinol *et al.* [33] demonstrated that oxysterols at concentrations higher than 1 μg/ml mediated the apoptotic cytotoxicity of oxidized low density lipoprotein, increasing intracellular [$^{45}Ca^{2+}$] in minutes. The increase in intracellular Ca^{2+} was prevented by the Ca^{2+} channel blocker nifedipine, and the authors concluded that oxysterols affect membrane Ca^{2+} channels. However, the nifedipine-sensitive channels (dihydropyridine receptor or L-type Ca^{2+} channels) are also major channels for replenishing SR Ca^{2+}. Therefore, by inhibiting the major membrane Ca^{2+} input by nifedipine, the authors significantly reduced the SR Ca^{2+} load.

Although cell membranes can accumulate oxysterols, and at high concentrations (~100 μM) oxysterols induce a lactate dehydrogenase leak through cardiac membranes [27], it is very doubtful that they affect voltage-dependent dihydropyridine-sensitive Ca^{2+} channels [97]. Indeed, Ares *et al.* [15] demonstrated that although the effects of 7β-hydroxycholesterol were dependent on extracellular [Ca^{2+}], verapamil, an inhibitor of Ca^{2+} channels, did not prevent the effects. The short-term (minutes) application of 25-hydroxycholesterol or 7β-hydroxycholesterol to aortic smooth muscle cells induced a significant increase in $[Ca^{2+}]_{cyt}$, [Ca^{2+}] oscillations and the emptying of the SR of Ca^{2+} [14,15]. This supported older data presented by Zhou *et al.* [32]. In 1991 they demonstrated a significant inhibition of SERCA activity by 25-hydroxycholesterol in bovine arterial smooth muscle cells [32].

Our recent experiments in ventricular myocytes demonstrated that within minutes both 7β- and 25-hydroxycholesterol significantly inhibited cell responses to electrical stimulations, increased resting cytoplasmic $[Ca^{2+}]_{cyt}$, reduced the SR Ca^{2+} content and frequency of Ca^{2+} sparks, and slowed down SR Ca^{2+} uptake by SR microsomes [7]. Based on our reports [38,53], we can explain these oxysterol effects in cardiac myocytes only by an inhibition of SERCA. Indeed, inhibition of SERCA has to lead to long-term Ca^{2+} emptying of the SR and a temporary increase in $[Ca^{2+}]_{cyt}$ [38]. It is doubtful that this short-term increase in $[Ca^{2+}]_{cyt}$ will induce ultrastructural remodeling and/or death of cardiac myocytes because drastic increases in $[Ca^{2+}]_{cyt}$ during contractions are normal for their physiology. However, a long-term reduction in the SR Ca^{2+} load was demonstrated to lead to a long-term increase in levels of cytosolic Ca^{2+} due to an increase in import of extracellular Ca^{2+} through transient receptor potential (TRP) channels and L-type Ca^{2+} channels (DHPR) followed their compensatory overexpression [98,99]. Therefore, an increase in $[Ca^{2+}]_{cyt}$ could be a major factor inducing oxysterol-related changes in morphology (remodeling) of cardiomyocytes and/or cell death.

Effects of oxysterols on cell morphology

Long-term (hours) exposure to oxysterols was demonstrated to cause alterations in cell morphology [14,15,92] and cell death [2,16,34,89–91]. There are three types of cell death with characteristic morphological aspects: type 1 (apoptosis); type 2 (autophagy) and type 3 (oncosis or necrosis) [100]. In different cell types oxysterols induce both apoptosis and oncosis (necrosis) [3,16,101,102]. Mechanisms of apoptosis induced by oxysterols and the proportions of cells exhibiting apoptotic or oncotic responses depend on the cell type and the particular oxysterol. It is important to note that some oxysterols are capable of inflicting oncotic death by lysis in a matter of hours [16].

The type of muscle cell death associated with oxysterol treatment tends to be apoptotic (i.e., has more apoptic than oncotic changes) [101]. However, autophagic cell death of human aortic smooth muscle cells treated with 7-ketocholesterol has also been reported [103]. The first changes in smooth muscle cell ultrastructure were visible within 4 h of incubation with 25 μM 25-hydroxycholesterol [14]: chromatin condensation, an increased number of lysosomes and the emergence of arrays of tubular

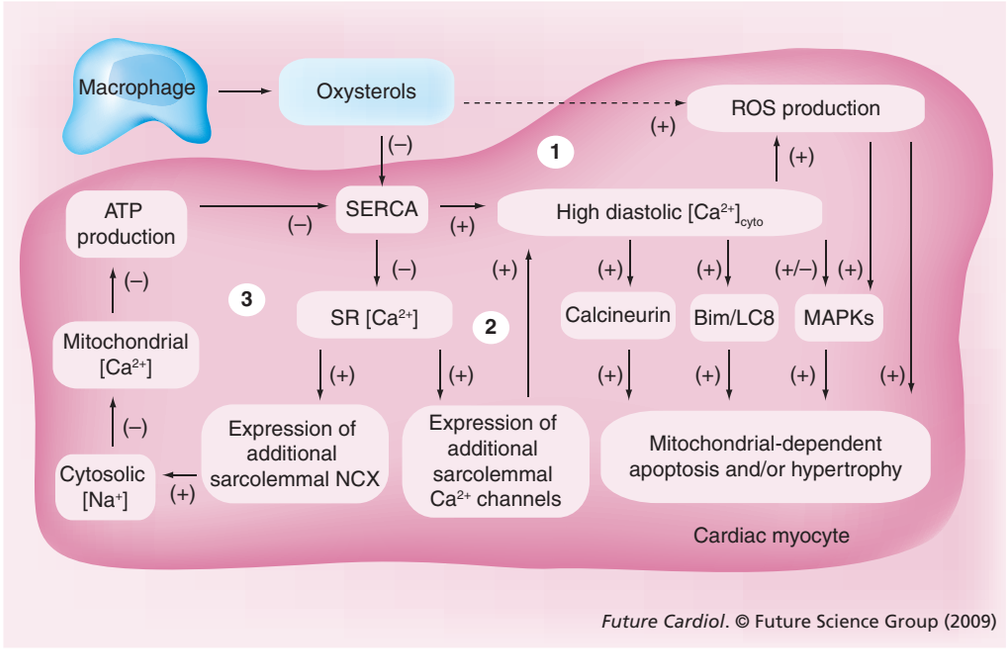

Figure 4. Putative pathways for oxysterol effects on cardiomyocytes leading to their structural remodeling and/or death. Numbers in circles mark three waves of elevation of $[Ca^{2+}]$ in cytoplasm. Bim/LC8: Stable stoichiometric complex of Bim and LC8; MAPK: Mitogen-activated protein kinase; NCX: Na^+/Ca^{2+} exchanger; ROS: Reactive oxygen species; SR: Sarcoplasmic reticulum.

membrane structures. After 24 h of treatment by different oxysterols, the cells began to lose ordered SR and Golgi membranes and to display blebs [14,15]. General swelling, a distinct feature of oncotic cell death, was not observed in the cells treated with oxysterols. It is not known whether the apparent loss of organized SR and Golgi membranes is a characteristic of apoptosis in oxysterol-induced cell death. However, swelling does not seem to be a dominant feature of these changes. Thus, despite the lack of some apoptotic features (such as budding and karyorhexis) oxysterol-induced cell death looks more apoptotic than oncotic. Recently, using *in situ* DNA fragmentation assay, Saito *et al.* [35] confirmed this finding in vascular smooth muscle cells.

Future perspective

Gaps in our understanding of the mechanisms involved in atherogenesis

Although coronary heart disease, ischemic heart disease, heart infarction and heart failure are among the most common complications of atherosclerosis, the specific mechanisms inducing the complications remain unknown. The primary consequence hypothesis that links elevated cholesterol to such dysfunction has been vascular, mainly focusing on the development of atherosclerotic changes in the local blood flow [78]. However, recent advances in the basic sciences have established a fundamental role for inflammation in mediating all stages of atherosclerosis from initiation through progression and, ultimately, the thrombotic complications of atherosclerosis. These new findings provide important links between risk factors and the mechanisms of atherogenesis [78].

Elevation of inflammation markers predicts the outcomes of patients with acute coronary syndromes, and certain treatments that reduce coronary risk are also known to reduce inflammation. However, in the case of lowering lipids with statins, this anti-inflammatory effect does not appear to correlate with a reduction in LDL levels [78] (see [2]). While theoretically compelling, and supported by a considerable body of experimental evidence, the relevance of the LDL oxidation hypothesis to human atherosclerosis remains unproven [78]. Thus, one of the unresolved issues is the relationship between LDL oxidation and the inflammatory theories in explaining the complications of atherosclerosis. However, LDL oxidation and inflammation are two facets of a single pathogenesis and we suggest that macrophage oxysterols can link these hypotheses [2]. Indeed, oxysterols could be produced in the inflammatory cells from oxidation of cholesterol. These kinds of oxysterols have been demonstrated

to freely defuse from macrophages and could affect multiple cell functions near the atherosclerotic core region.

Analysis of the literature and our preliminary data allow us to suggest that the major pathway for the effect of oxysterols involves an increase in diastolic [Ca^{2+}] in the cytoplasm of cardiomyocytes through Ca^{2+} empting of the SR followed by compensatory expression of additional Ca^{2+} channels into sarcolemma [7]. Figure 4 demonstrates the putative pathways for the effect of oxysterols leading to cardiomyocyte malfunction and death. Early on, macrophages create a local but deleterious concentration of oxysterols. These diffuse into cardiomyocytes, induce a reduction in SERCA activity and increase cytoplasmic [Ca^{2+}]. The effectiveness of this elevation of cytoplasmic [Ca^{2+}] depends on the oxysterol concentration and the timing. It is very doubtful that this first wave of cytoplasmic Ca^{2+} (marked with 1 in the circle) will be directly involved in cardiomyocyte remodeling and/or death. However, in the case of sustained exposure to oxysterols, it could activate mechanisms leading to cell hypertrophy and/or death. First of all, Ca^{2+} could activate production of reactive oxygen species (ROS). Although some authors are skeptical regarding the role for oxysterol-induced ROS in atherosclerosis [78], others consider ROS to be a major factor in oxysterol-related cell damage [3]. Indeed, although low levels of ROS drive diverse and important cellular functions, excessive ROS generation leads to both apoptotic and oncotic cell death [104–105]. This effect of oxysterols on ROS production was demonstrated to be mediated by high mitochondrial [Ca^{2+}] [105,107,108], which results from an increase in cytoplasmic [Ca^{2+}] [109]. However, mechanisms of initiation of apoptosis by ROS are not clear yet. ROS can: first, directly injure cell membranes and cause cell death; second, enhance the disruption of the mitochondrial membrane due to mitochondrial Ca^{2+} overload; or third, induce apoptosis associated with activation of MAPKs [108,110–113]. Considerable work needs to be done in order to understand the relationship between ROS, oxysterols and calcium signaling in heart disease, but the initial evidence is exciting.

Another way to induce cell hypertrophy and/or death could depend on subsequent waves of increased cytoplasmic [Ca^{2+}]. Corresponding pathways are marked with numbers in circles on Figure 4. Oxysterol-induced SR Ca^{2+} emptying activates compensatory expression of sarcolemmal Ca^{2+} channels and Na^+/Ca^{2+} exchangers (NCX) [98,99]. According to Seth *et al.* [98], the former significantly increases diastolic cytosolic [Ca^{2+}]. That should increase SR [Ca^{2+}] until normal values are reached; however, the latter (overexpression of sarcolemmal NCX) results in extensive Na^+ input through Na^+/Ca^{2+} exchangers that can significantly reduce mitochondrial [Ca^{2+}] and, consequently, ATP production [109,114]. Lack of ATP has to reduce SR [Ca^{2+}] and results in additional increases in the levels of cytosolic [Ca^{2+}]. If the increase in cytoplasmic [Na^+] is not found sufficient to start the compensatory expression cycle again (and another wave of Ca^{2+} elevation) it could be important in preventing a Ca^{2+}-induced permeability transition, which results in swelling and disruption of cardiac mitochondria and release of cytochrome c [115].

In addition to but independent of the process of increased long-term $[Ca^{2+}]_{cyt}$, high $[Ca^{2+}]_{cyt}$ can induce heart remodeling and death

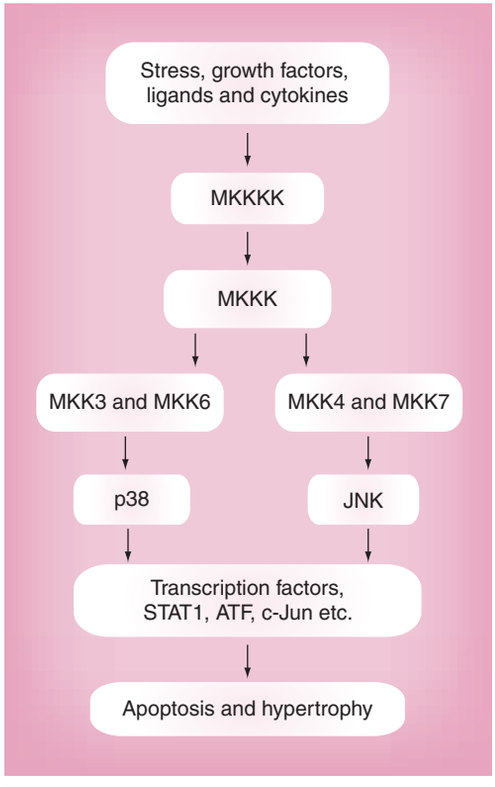

Figure 5. Representation of the activation of MAPKs, leading to cell hypertrophy and/or death. ATF: Activating transcription factor; c-Jun: Transcription factor; JNK: c-Jun NH2-terminal kinase; MKK: Mitogen-activated protein kinase kinase; MKKK: Mitogen-activated protein kinase kinase kinase; MKKKK: Mitogen-activated protein kinase kinase kinase kinase; p38: Mitogen-activated protein kinase p38; STAT: Signal transducer and activator of transcription protein.

of cardiomyocytes through three other pathways (Figure 4): first, activation of calcineurin, which is involved in activation of compensatory expression cycle [98,99], but also induce mitochondria-dependent apoptosis [3]; second, activation of some proapoptic MAPKs and inhibition of antiapoptic MAPKs; and third, promotion of proapoptotic Bim/LC8 unbinding from the dynein motor complex and relocation to the mitochondria, where it interacts with Bcl-2 [34,116]. Which of these three pathways is most harmful for cardiac cells remains to be elucidated. So far, the direct mechanisms underlying the long-term effects of oxysterols have not been investigated substantially in the heart. There is only one paper describing such an effect of cholesterol 5α,6α-epoxide and only on neonatal cardiac cells [27]. However, the data presented in the article only supported the existence of a direct toxic effect of oxysterols on cardiac myocytes. Meanwhile, some indirect data suggest that oxysterols may play a significant role in the development of cardiomyopathy [30].

Most probable candidates for connecting long-term & rapid effects of oxysterols on cardiac cells

The long-term (mainly morphological) and rapid effects of oxysterols must be connected. In our opinion, the most probable candidates for connecting them are Ca^{2+} and MAPKs [15]. Indeed, the fastest effect of oxysterols is to induce an increase in $[Ca^{2+}]_{cyt}$, and a similar increase in $[Ca^{2+}]_{cyt}$ was demonstrated to affect the activity of all three branches of the MAPK signaling pathway: c-Jun N-terminal kinase (JNK), p38, and extracellular signal-regulated kinase (ERK) [15,117]. Berthier *et al.* [34] demonstrated that the activation of ERK by oxysterols is mediated by the Ca^{2+}-dependent tyrosine kinase PYK2. Activation or inhibition of certain MAPKs (Figure 5) were demonstrated to be responsible for impairing cardiac contractile function, inducing heart hypertrophy and causing apoptotic cell death [5,82–85,87]. One can suggest that like other cell types, an oxysterol-induced increase in $[Ca^{2+}]_{cyt}$ should also influence the activity of MAPKs in cardiac myocytes. MAPKs (p38 and JNK) were demonstrated to play significant role in apoptosis [5,85].

Another way for oxysterols to induce cell death is through the depletion of thapsigargin-sensitive Ca^{2+} stores (i.e., SR) [15]. Indeed, low SR Ca^{2+} load itself was shown to induce apoptosis. There are two hypotheses that can explain the phenomenon of thapsigargin-induced apoptosis [118]. Long-term depletion of the SR Ca^{2+} pool might result in: first, disruption of protein processing and transport within the SR/ER; and second, the release of an endonuclease responsible for DNA fragmentation. Alternative pathway for downregulation of protein synthesis leading to cell death is mitochondrial Ca^{2+} overload [115,119]. Thus, the mechanisms connecting oxysterol-induced SERCA inhibition and cell-death remain to be elucidated.

Future perspective

Future research is needed to clarify the mechanisms of atherosclerosis-related contractility disorders in the heart, as well as the pathways leading to atherosclerosis-induced cell death in all tissues located in close proximity to the atherosclerotic core region. New insights into the role of oxysterols in the development of heart complications of atherosclerosis will not only increase our understanding of inflammation-induced heart complications and atherosclerosis progression, but will have practical clinical applications in risk stratification, the targeting of known therapies for the disease, and the development of new therapeutic strategies.

Conclusion

New findings suggest that the heart muscle can develop dysfunctional Ca^{2+} cycling leading to cell death as a result of elevated serum cholesterol derivatives, the oxysterols. This means that oxysterols could be one of the major factors precipitating morbidity in atherosclerosis-induced cardiac diseases and inflammation-induced heart complications. The analysis presented here of current literature permits us to suggest that oxysterols could induce dysfunction of cardiac cells through their effects on the cardiac SR Ca^{2+} signaling. Therefore, major efforts should be undertaken in the near future to elucidate the effects of oxysterols on: Ca^{2+} handling, Ca^{2+}-dependent activity of mitogen-activated protein kinases (MAPKs), and MAPK-related changes of cell ultrastructure in cardiomyocytes.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Executive summary

Oxysterols

- Oxysterols were demonstrated to have pathological effects on the cells.
- Oxysterols can be produced by macrophages and diffuse to the neighbor cells.

Cardiac Ca^{2+} signaling

- Modulation of sarcoplasmic reticulum (SR) Ca^{2+} cycling mechanisms results in corresponding changes in the SR Ca^{2+} load, cytosolic Ca^{2+} and spontaneous SR Ca^{2+} release.
- Defective SR Ca^{2+} cycling is responsible for defective excitation-contraction coupling in heart failure.

Atherosclerosis

- Production of oxysterols occurs in atherosclerotic plaques.
- Although functional effects of oxysterols on a number of cell types have been described, their role in the morbidity of atherosclerosis-related cardiac diseases remains unknown.

Heart failure

- Inflammatory processes are directly involved in cardiac depression.
- Macrophage oxysterols could be involved in the pathogenesis of early stages of heart failure.
- Abnormalities in Ca^{2+} handling during heart failure are similar to those caused by oxysterols in muscle cells.

Oxysterol-induced changes in cell ultrastructure & function

- There are fast and long-term effects of oxysterols.
- Oxysterols induce a fast Ca^{2+} emptying of the SR.
- The type of cell death associated with oxysterol treatment tends to be apoptotic.

Future perspective

- Gaps in our understanding of the mechanisms involved in atherogenesis.
- The most probable candidates for connecting long-term and fast effects of oxysterols are Ca^{2+} and mitogen-activated protein kinases.

Bibliography

Papers of special note have been highlighted as:
- of interest

1. Zingg JM, Ricciarelli R, Azzi A: Scavenger receptor regulation and atherosclerosis. *Biofactors* 11, 189–200 (2000).
2. Steinberg D: Atherogenesis in perspective: hypercholesterolemia and inflammation as partners in crime. *Nat. Med.* 8, 1211–1217 (2002).
3. Vejux A, Malvitte L, Lizard G: Side effects of oxysterols: cytotoxicity, oxidation, inflammation, and phospholipidosis. *Braz. J. Med. Biol. Res.* 41, 545–556 (2008).

▪ **One of the best reviews about oxysterols.**

4. Schroepfer GJ Jr: Oxysterols: modulators of cholesterol metabolism and other processes. *Physiol. Rev.* 80, 361–554 (2000).

▪ **Best review about oxysterols.**

5. Muslin AJ: MAPK signalling in cardiovascular health and disease: molecular mechanisms and therapeutic targets. *Clin. Sci.* 115, 203–218 (2008).
6. Seimon T, Tabas I: Mechanisms and consequences of macrophage apoptosis in atherosclerosis. *J. Lipid Res.* 50, S382–S387 (2009).
7. Lukyanenko V, Lederer WJ: Effects of oxysterols on the SR Ca^{2+} cycling in ventricular myocytes. *Biophys. J.* 94, 514a (2009).
8. Brown AJ, Jessup W: Oxysterols and atherosclerosis. *Atherosclerosis* 142, 1–28 (1999).
9. Bjorkhem I, Andersson O, Diczfalusy U *et al.*: Atherosclerosis and sterol 27-hydroxylase: evidence for a role of this enzyme in elimination of cholesterol from human macrophages. *Proc. Natl Acad. Sci. USA* 91, 8592–8596 (1994).
10. Hessler JR, Morel DW, Lewis LJ, Chisolm GM: Lipoprotein oxidation and lipoprotein-induced cytotoxicity. *Arteriosclerosis* 3, 215–222 (1983).
11. Hulten LM, Lindmark H, Diczfalusy U *et al.*: Oxysterols present in atherosclerotic tissue decrease the expression of lipoprotein lipase messenger RNA in human monocyte-derived macrophages. *J. Clin. Invest.* 97, 461–468 (1996).
12. Peng SK, Hu B, Morin RJ: Angiotoxicity and atherogenicity of cholesterol oxides. *J. Clin. Lab. Anal.* 5, 144–152 (1991).
13. Liu Y, Hulten LM, Wiklund O: Macrophages isolated from human atherosclerotic plaques produce IL-8, and oxysterols may have a regulatory function for IL-8 production. *Arterioscler. Thromb. Vasc. Biol.* 17, 317–323 (1997).
14. Ares MP, Pörn-Ares MI, Thyberg J *et al.*: Ca^{2+} channel blockers verapamil and nifedipine inhibit apoptosis induced by 25-hydroxy-cholesterol in human aortic smooth muscle cells. *J. Lipid Res.* 38, 2049–2061 (1997).
15. Ares MP, Pörn-Ares MI, Moses S *et al.*: 7β-hydroxycholesterol induces Ca^{2+} oscillations, MAP kinase activation and apoptosis in human aortic smooth muscle cells. *Atherosclerosis* 153, 23–35 (2000).
16. Colles SM, Maxson JM, Carlson SG, Chisolm GM: Oxidized LDL-induced injury and apoptosis in atherosclerosis. Potential roles for oxysterols. *Trends Cardiovasc. Med.* 11, 131–138 (2001).
17. Zwijsen RM, Oudenhoven IM, de Haan LH: Effects of cholesterol and oxysterols on gap junctional communication between human smooth muscle cells. *Eur. J. Pharmacol.* 228, 115–120 (1992).
18. Lund E, Andersson O, Zhang J *et al.*: Importance of a novel oxidative mechanism for elimination of intracellular cholesterol in humans. *Arterioscler. Thromb. Vasc. Biol.* 16, 208–212 (1996).
19. van Reyk DM, Jessup W: The macrophage in atherosclerosis: modulation of cell function by sterols. *J. Leukoc. Biol.* 66, 557–561 (1999).
20. Nes WD, Lukyanenko Y, Jia ZH *et al.*: Identification of the lipophilic factor produced by macrophages that stimulates steroidogenesis. *Endocrinol.* 141, 953–958 (2000).
21. Lund EG, Kerr TA, Sakai J, Li WP, Russell DW: cDNA cloning of mouse and human cholesterol 25-hydroxylases, polytopic membrane proteins that synthesize a potent oxysterol regulator of lipid metabolism. *J. Biol. Chem.* 273, 34316–34327 (1998).

22. Johnson KA, Morrow CJ, Knight GD, Scallen TJ: *In vivo* formation of 25-hydroxycholesterol from endogenous cholesterol after a single meal, dietary cholesterol challenge. *J. Lipid Res.* 35, 2241–2253 (1994).
23. Zarev S, Therond P, Bonnefont-Rousselot D *et al.*: Major differences in oxysterol formation in human low density lipoproteins (LDLs) oxidized by $\cdot OH/O_2^{\cdot -}$ free radicals or by copper. *FEBS Lett.* 451, 103–108 (1999).
24. Shanahan CM, Carpenter KL, Cary NR: A potential role for sterol 27-hydroxylase in atherogenesis. *Atherosclerosis* 154, 269–276 (2001).
25. Harvey EJ, Ramji DP: Interferon-γ and atherosclerosis: pro- or anti-atherogenic? *Cardiovasc. Res.* 67, 11–20 (2005).
26. Lukyanenko Y, Carpenter AM, Brigham DE, Stocco DM, Hutson JC: Regulation of Leydig cells through a steroidogenic acute regulatory protein-independent pathway by a lipophilic factor from macrophages. *J. Endocrinol.* 158, 267–275 (1998).
27. Hrelia S, Bordoni A, Caboni MF *et al.*: Effect of cholesterol-5 α,6 α-epoxide supplementation to cultured cardiomyocytes. *Biochem. Mol. Biol. Int.* 32, 565–573 (1994).
28. Li S, Pang J, Jackson EM, Wilson WK, Mott GE, Schroepfer GJ Jr: Kinetics and plasma concentrations of 26-hydroxycholesterol in baboons. *Biochim. Biophys. Acta* 1485, 173–184 (2000).
29. Zhou Q, Wasowicz E, Handler B, Fleischer L, Kummerow FA: An excess concentration of oxysterols in the plasma is cytotoxic to cultured endothelial cells. *Atherosclerosis* 149, 191–197 (2000).
30. Matsui H, Okumura K, Mukawa H *et al.*: Increased oxysterol contents in diabetic rat hearts: their involvement in diabetic cardiomyopathy. *Can. J. Cardiol.* 13, 373–379 (1997).
31. Yoshioka N, Adachi J, Ueno Y, Yoshida K: Oxysterols increase in diabetic rats. *Free Radic. Res.* 39, 299–304 (2005).
32. Zhou Q, Jimi S, Smith T, Kummerow F: The effect of 25-hydroxycholesterol on accumulation of intracellular calcium. *Cell Calcium* 12, 467–476 (1991).
33. Rusinol AE, Yang L, Thewke D *et al.*: Isolation of a somatic cell mutant resistant to the induction of apoptosis by oxidized low density lipoprotein. *J. Biol. Chem.* 275, 7296–7303 (2000).
34. Berthier A, Lemaire-Ewing S, Prunet C *et al.*: 7-Ketocholesterol-induced apoptosis. Involvement of several pro-apoptotic but also anti-apoptotic calcium-dependent transduction pathways. *FEBS J.* 272, 3093–3104 (2005).
35. Saito E, Wachi H, Sato F, Seyama Y: 7-ketocholesterol, a major oxysterol, promotes pi-induced vascular calcification in cultured smooth muscle cells. *J. Atheroscler. Thromb.* 15, 130–137 (2008).
36. Eisner DA, Choi HS, Diaz ME, O'Neill SC, Trafford AW: Integrative analysis of calcium cycling in cardiac muscle. *Circ. Res.* 87, 1087–1094 (2000).
37. Bers DM: *Excitation–Contraction Coupling and Cardiac Contractile Force.* Kluwer Academic Publishers, Dordrecht, The Netherlands (2001).

▪ **This is the best book about excitation–contraction coupling in cardiac myocytes.**

38. Lukyanenko V, Viatchenko-Karpinski S, Smirnov A, Wiesner TF, Györke S: Dynamic regulation of the SR Ca^{2+} content and release by lumenal Ca^{2+}-sensitive leak through RyRs in rat ventricular myocytes. *Biophys. J.* 81, 785–798 (2001).
39. Feher JJ, Fabiato A. Cardiac sarcoplasmic reticulum: calcium uptake and release. In: *Calcium and Heart.* Langer GA (Ed.). Raven Press, Ltd, NY, USA, 199–267 (1990).
40. Chiesi M, Wrzosek A, Grueninger S: The role of the sarcoplasmic reticulum in various types of cardiomyocytes. *Mol. Cell Biochem.* 130, 159–171 (1994).
41. Fabiato A: Time and calcium dependence of activation and inactivation of calcium-induced calcium release of calcium from the sarcoplasmic reticulum of a skinned canine cardiac Purkinje cell. *J. Gen. Physiol.* 85, 291–320 (1985).
42. Cannell MB, Cheng H, Lederer WJ: The control of calcium release in heart muscle. *Science* 268, 1045–1049 (1995).
43. Marx SO, Reiken S, Hisamatsu Y *et al.*: PKA phosphorylation dissociates FKBP12.6 from the calcium release channel (ryanodine receptor): defective regulation in failing hearts. *Cell* 101, 365–376 (2000).
44. Bers DM: Cardiac excitation–contraction coupling. *Nature* 415, 198–205 (2002).
45. Lukyanenko V, Györke I, Györke S: Regulation of calcium release by calcium inside the sarcoplasmic reticulum in ventricular myocytes. *Pflug. Arch.* 432, 1047–1054 (1996).
46. Lukyanenko V, Györke S: Ca^{2+} sparks and Ca^{2+} waves in saponin-permeabilized cardiac myocytes. *J. Physiol.* 521, 575–585 (1999).
47. Cheng H, Lederer W, Cannell M: Calcium sparks: elementary events underlying excitation-contraction coupling in heart muscle. *Science* 262, 740–744 (1993).
48. Lopez-Lopez J, Shacklock P, Balke CW, Wier WG: Local calcium transients triggered by single L-type calcium channel currents in cardiac cells. *Science* 268, 1042–1045 (1995).
49. Lukyanenko V, Györke I, Subramanian S *et al.*: Inhibition of Ca^{2+} sparks by ruthenium red in permeabilized rat ventricular myocytes. *Biophys. J.* 79, 1273–1284 (2000).
50. Cannell MB, Cheng H, Lederer WJ: Spatial non-uniformities in $[Ca^{2+}]_i$ during excitation-contraction coupling in cardiac myocytes. *Biophys. J.* 67, 1942–1956 (1994).
51. Wang SQ, Song LS, Lakatta EG, Cheng H: Ca^{2+} signalling between single L-type Ca^{2+} channels and ryanodine receptors in heart cells. *Nature* 410, 592–596 (2001).
52. Izu LT, Wier WG, Balke CW: Evolution of cardiac calcium waves from stochastic calcium sparks. *Biophys. J.* 80, 103–120 (2001).
53. Lukyanenko V, Györke I, Wiesner TF, Györke S: Potentiation of Ca^{2+} release by cADPR in heart is mediated by enhanced Ca^{2+} uptake into the sarcoplasmic reticulum. *Circ. Res.* 89, 614–622 (2001).
54. Schmidt U, Hajjar RJ, Helm PA, Kim CS, Doye AA, Gwathmey JK: Contribution of abnormal sarcoplasmic reticulum ATPase activity to systolic and diastolic dysfunction in human heart failure. *J. Mol. Cell Cardiol.* 30, 1929–1937 (1998).
55. Currie S, Smith GL: Enhanced phosphorylation of phospholamban and downregulation of sarco/endoplasmic reticulum Ca^{2+} ATPase type 2 (SERCA2) in cardiac sarcoplasmic reticulum from rabbits with heart failure. *Cardiovasc. Res.* 41, 135–146 (1999).
56. Kirchhefer U, Schmitz W, Scholz H, Neumann J: Activity of cAMP-dependent protein kinase and Ca^{2+}/calmodulin-dependent protein kinase in failing and nonfailing human hearts. *Cardiovasc. Res.* 42, 254–261 (1999).
57. Haghighi K, Schmidt AG, Hoit BD *et al.*: Superinhibition of sarcoplasmic reticulum function by phospholamban induces cardiac contractile failure. *J. Biol. Chem.* 276, 24145–24152 (2001).
58. Hobai IA, O'Rourke B: Decreased sarcoplasmic reticulum calcium content is responsible for defective excitation-contraction coupling in canine heart failure. *Circulation* 103, 1577–1584 (2001).

59. Hasenfuss G, Pieske B: Calcium cycling in congestive heart failure. *J. Mol. Cell. Cardiol.* 34, 951–969 (2002).
60. Lehnart SE, Terrenoire C, Reiken S *et al.*: Stabilization of cardiac ryanodine receptor prevents intracellular calcium leak and arrhythmias. *Proc. Natl Acad. Sci. USA* 103, 7906–7910 (2006).
61. Lehnart SE, Mongillo M, Bellinger A *et al.*: Leaky Ca^{2+} release channel/ryanodine receptor 2 causes seizures and sudden cardiac death in mice. *J. Clin. Invest.* 118, 2230–2245 (2008).
62. Maier LS, Bers DM: Role of Ca^{2+}/calmodulin-dependent protein kinase (CaMK) in excitation-contraction coupling in the heart. *Cardiovasc. Res.* 73, 631–640 (2007).
63. Ferrier GR: The effects of tension on acetylstrophanthidin-induced transient depolarizations and aftercontractions in canine myocardial and Purkinje tissues. *Circ. Res.* 38, 156–162 (1976).
64. Kihara Y, Morgan JP: Intracellular calcium and ventricular fibrillation. Studies in the aequorin-loaded isovolumic ferret heart. *Circ. Res.* 68, 1378–1389 (1991).
65. Lakatta EG: Functional implications of spontaneous sarcoplasmic reticulum Ca^{2+} release in the heart. *Cardiovasc. Res.* 26, 193–214 (1992).
66. Ishide N: Intracellular calcium modulators for cardiac muscle in pathological conditions. *Jap. Heart J.* 37, 1–17 (1996).
67. Carmeliet E: Cardiac ionic currents and acute ischemia: from channels to arrhythmias. *Physiol. Rev.* 79, 917–1017 (1999).
68. Janse MJ: Electrophysiology of arrhythmias. *Arch. Mal. Coeur. Vaiss.* 92, 9–16 (1999).
69. Marks AR: Ryanodine receptors/calcium release channels in heart failure and sudden cardiac death. *J. Mol. Cell. Cardiol.* 33, 615–624 (2001).
70. Pogwizd SM, Bers DM: Calcium cycling in heart failure: the arrhythmia connection. *J. Cardiovasc. Electrophysiol.* 13, 88–91 (2002).
71. Bellinger AM, Reiken S, Dura M *et al.*: Remodeling of ryanodine receptor complex causes 'leaky' channels: a molecular mechanism for decreased exercise capacity. *Proc. Natl Acad. Sci. USA* 105, 2198–2202 (2008).
72. Ross R: Cell biology of atherosclerosis. *Annu. Rev. Physiol.* 57, 791–804 (1995).
73. Schmermund A, Schwartz RS, Adamzik M *et al.*: Coronary atherosclerosis in unheralded sudden coronary death under age 50: histo-pathologic comparison with 'healthy' subjects dying out of hospital. *Atherosclerosis* 155, 499–508 (2001).
74. Tomaselli GF, Zipes DP: What causes sudden death in heart failure? *Circ. Res.* 95, 754–763 (2004).
75. Steffens S, Veillard NR, Arnaud C *et al.*: Low dose oral cannabinoid therapy reduces progression of atherosclerosis in mice. *Nature* 434, 782–786 (2005).
76. Mehta JL, Li DY: Inflammation in ischemic heart disease: response to tissue injury or a pathogenetic villain? *Cardiovasc. Res.* 43, 291–299 (1999).
77. Lusis AJ: Atherosclerosis. *Nature* 407, 233–241 (2000).
78. Libby P, Ridker PM, Maseri A: Inflammation and atherosclerosis. *Circulation* 105, 1135–1143 (2002).
79. Maxwell SR: Can anti-oxidants prevent ischaemic heart disease? *J. Clin. Pharm. Ther.* 18, 85–95 (1993).
80. Houser SR, Margulies KB: Is depressed myocyte contractility centrally involved in heart failure? *Circ. Res.* 92, 350–358 (2003).
81. Blum A, Miller H: Pathophysiological role of cytokines in congestive heart failure. *Annu. Rev. Med.* 52, 15–27 (2001).
82. Liao P, Wang SQ, Wang S *et al.*: p38 Mitogen-activated protein kinase mediates a negative inotropic effect in cardiac myocytes. *Circ. Res.* 90, 190–196 (2002).
83. Andrews C, Ho PD, Dillmann WH, Glembotski CC, McDonough PM: The MKK6-p38 MAPK pathway prolongs the cardiac contractile calcium transient, downregulates SERCA2, and activates NF-AT. *Cardiovasc. Res.* 59, 46–56 (2003).
84. Wold LE, Ren J: Streptozotocin directly impairs cardiac contractile function in isolated ventricular myocytes via a p38 map kinase-dependent oxidative stress mechanism. *Biochem. Biophys. Res. Commun.* 318, 1066–1071 (2004).
85. Baines CP, Molkentin JD: STRESS signaling pathways that modulate cardiac myocyte apoptosis. *J. Mol. Cell. Cardiol.* 38, 47–62 (2005).
86. Beuckelmann DJ, Nabauer M, Erdmann E: Intracellular calcium handling in isolated ventricular myocytes from patients with terminal heart failure. *Circulation* 85, 1046–1055 (1992).
87. Zarubin T, Han J: Activation and signaling of the p38 MAP kinase pathway. *Cell Res.* 15, 11–18 (2005).
88. Lemaire-Ewing S, Prunet C, Montange T *et al.*: Comparison of the cytotoxic, pro-oxidant and pro-inflammatory characteristics of different oxysterols. *Cell Biol Toxicol.* 21, 97–114 (2005).
89. Hughes H, Mathews B, Lenz ML, Guyton JR: Cytotoxicity of oxidized LDL to porcine aortic smooth muscle cells is associated with the oxysterols 7-ketocholesterol and 7-hydroxycholesterol. *Arterioscler. Thromb.* 14, 1177–1185 (1994).
90. Guyton JR, Black BL, Seidel CL: Focal toxicity of oxysterols in vascular smooth muscle cell culture. A model of the atherosclerotic core region. *Am. J. Pathol.* 137, 425–434 (1990).
91. Zhou Q, Wasowicz E, Kummerow FA: Failure of vitamin E to protect cultured human arterial smooth muscle cells against oxysterol-induced cytotoxicity. *J. Am. Coll. Nutr.* 14, 169–175 (1995).
92. Wohlfeil ER, Campbell WB: 25-hydroxycholesterol increases eicosanoids and alters morphology in cultured pulmonary artery smooth muscle and endothelial cells. *Arterioscler. Thromb. Vasc. Biol.* 19, 2901–2908 (1999).
93. Yang L, Sinensky MS: 25-Hydroxycholesterol activates a cytochrome c release-mediated caspase cascade. *Biochem. Biophys. Res. Commun.* 278, 557–563 (2000).
94. Choi YK, Kim YS, Choi IY, Kim SW, Kim WK: 25-hydroxycholesterol induces mitochondria-dependent apoptosis via activation of glycogen synthase kinase-3β in PC12 cells. *Free Radic. Res.* 42, 544–553 (2008).
95. Glass CK, Saijo K: Oxysterols hold T cells in check. *Nature* 455, 40–41 (2008).
96. Peng SK, Hill JC, Morin RJ, Taylor CB: Influence of cholesterol oxidation derivatives on membrane bound enzymes in cultured aortic smooth muscle cells. *Proc. Soc. Exp. Biol. Med.* 180, 126–1321 (1985).
97. Chang JY, Phelan KD, Chavis JA: Neurotoxicity of 25-Hydroxycholesterolesterol on sympathetic neurons. *Brain Res. Bull.* 45, 615–622 (1998).
98. Seth M, Sumbilla C, Mullen SP *et al.*: Sarco(endo)plasmic reticulum Ca^{2+} ATPase (SERCA) gene silencing and remodeling of the Ca^{2+} signaling mechanism in cardiac myocytes. *Proc. Natl Acad. Sci. USA* 101, 16683–16688 (2004).
99. Andersson KB, Birkeland JA, Finsen AV *et al.*: Moderate heart dysfunction in mice with inducible cardiomyocyte-specific excision of the Serca2 gene. *J. Mol. Cell Cardiol.* (2009) (In press).
100. Galluzzi L, Maiuri MC, Vitale I *et al.*: Cell death modalities: classification and pathophysiological implications. *Cell Death Differ.* 14, 1237–1243 (2007).

▪ **Here you will find a modern classification of types of cell death.**

101. Lizard G, Monier S, Cordelet C *et al.*: Characterization and comparison of the mode of cell death, apoptosis versus necrosis, induced by 7β-hydroxycholesterol and 7-ketocholesterol in the cells of the vascular wall. *Arterioscler. Thromb. Vasc. Biol.* 19, 1190–1200 (1999).

102. Prunet C, Lemaire-Ewing S, Ménétrier F, Néel D, Lizard G: Activation of caspase-3-dependent and -independent pathways during 7-ketocholesterol- and 7β-hydroxycholesterol-induced cell death: a morphological and biochemical study. *J. Biochem. Mol. Toxicol.* 19, 311–326 (2005).

103. Miguet-Alfonsi C, Prunet C, Monier S *et al.*: Analysis of oxidative processes and of myelin figures formation before and after the loss of mitochondrial transmembrane potential during 7β-hydroxycholesterol and 7-ketocholesterol-induced apoptosis: comparison with various pro-apoptotic chemicals. *Biochem. Pharmacol.* 64, 527–541 (2002).

104. Dypbukt JM, Ankarcrona M, Burkitt M *et al.*: Different prooxidant levels stimulate growth, trigger apoptosis, or produce necrosis of insulin-secreting RINm5F cells. The role of intracellular polyamines. *J. Biol. Chem.* 269, 30553–30560 (1994).

105. Brookes PS, Yoon Y, Robotham JL, Anders MW, Sheu SS: Calcium, ATP, and ROS: a mitochondrial love-hate triangle. *Am. J. Physiol. Cell Physiol.* 287, C817–C833 (2004).

106. Wang W, Fang H, Groom L *et al.*: Superoxide flashes in single mitochondria. *Cell* 134, 279–290 (2008).

107. Duan Y, Gross RA, Sheu SS: Ca^{2+}-dependent generation of mitochondrial reactive oxygen species serves as a signal for poly(ADP-ribose) polymerase-1 activation during glutamate excitotoxicity. *J. Physiol.* 585, 741–758 (2007).

108. Murphy E, Steenbergen C: Mechanisms underlying acute protection from cardiac ischemia-reperfusion injury. *Physiol. Rev.* 88, 581–609 (2008).

109. Lukyanenko V, Chikando A, Lederer WJ: Calcium signaling in cardiac mitochondria and sarcoplasmic reticulum. *Int. J. Biochem. Cell Biol.* DOI:10.1016/j.biocel.2009.03.011 (2009) (In press).

110. Foncea R, Carvajal C, Almarza C, Leighton F: Endothelial cell oxidative stress and signal transduction. *Biol. Res.* 33, 89–96 (2000).

111. Yoon JH, Canbay AE, Werneburg NW, Lee SP, Gores GJ: Oxysterols induce cyclooxygenase-2 expression in cholangiocytes: implications for biliary tract carcinogenesis. *Hepatology* 39, 732–738 (2004).

112. Chen L, Liu L, Yin J, Luo Y, Huang S: Hydrogen peroxide-induced neuronal apoptosis is associated with inhibition of protein phosphatase 2A and 5, leading to activation of MAPK pathway. *Int. J. Biochem. Cell Biol.* 41, 1284–1295 (2008).

113. Hori M, Nishida K: Oxidative stress and left ventricular remodeling after myocardial infarction. *Cardiovasc. Res.* 81, 457–464 (2008).

114. Cortassa S, Aon MA, Marban E, Winslow RL, O'Rourke B: An integrated model of cardiac mitochondrial energy metabolism and calcium dynamics. *Biophys. J.* 84, 2734–2755 (2003)

115. Salnikov VV, Lukyanenko YO, Frederick CA, Lederer WJ, Lukyanenko V: Probing the outer mitochondrial membrane in cardiac mitochondria with nanoparticles. *Biophys. J.* 92, 1058–1071 (2007).

116. Puthalakath H, Huang DC, O'Reilly LA, King SM, Strasser A: The proapoptotic activity of the Bcl-2 family member Bim is regulated by interaction with the dynein motor complex. *Mol. Cell* 3, 287–296 (1999).

117. Kim J, Sharma RP: Calcium-mediated activation of c-Jun NH_2-terminal kinase (JNK) and apoptosis in response to cadmium in murine macrophages. *Toxicol. Sci.* 81, 518–527 (2004).

118. He H, Lam M, McCormick TS, Distelhorst CW: Maintenance of calcium homeostasis in the endoplasmic reticulum by Bcl-2. *J. Cell. Biol.* 138, 1219–1228 (1997).

119. Soboloff J, Berger SA: Sustained ER Ca^{2+} depletion suppresses protein synthesis and induces activation-enhanced cell death in mast cells. *J. Biol. Chem.* 277, 13812–13820 (2002).

120. Parfenov AS, Salnikov V, Lederer WJ, Lukyanenko V: Aqueous diffusion pathways as a part of the ventricular cell ultrastructure. *Biophys. J.* 9, 1107–1119 (2006).

Affiliations

- Valeriy Lukyanenko, PhD
Assistant Professor, Medical Biotechnology Center, University of Maryland Biotechnology Institute, 725 W. Lombard St, Rm S216, Baltimore, MD 21201, USA
Tel.: +1 410 706 8559
Fax: +1 410 706 8184
lukyanen@umbi.umd.edu

- Yevgeniya Lukyanenko
Research Associate, Department of Biochemistry & Molecular Biology, University of Maryland, School of Medicine, 108 N Greene Street, Baltimore, MD 21201, USA
Tel.: +1 410 706 5734
Fax: +1 410 706 6676
yluky001@umaryland.edu